

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: H. Outtrup *et al.* ) Attorney Docket No.:  
Serial Number: Not Yet Assigned ) VAL6131P0208AUS  
Filing Date: May 15, 2001 ) 5910.US.C4  
Re: Mutants Which Produce a Potentiator ) Group Art Unit: Not Yet Assigned  
of *Bacillus* Pesticidal Activity ) Examiner: Not Yet Assigned

Commissioner for Patents  
Washington, D.C., 20231

PRELIMINARY AMENDMENT

Dear Sir:

In connection with the filing of a continuation application from U.S. Patent Application Serial No. 09/013,375, please amend the application as follows. A Utility Application Transmittal Sheet, copies of two executed Declarations, a Declaration of Deposit, an IDS and a check for \$710.00 accompany this Preliminary Amendment.

IN THE SPECIFICATION

Please replace the paragraph beginning at page 1, line 6 with the following re-written paragraph. --Cross-Reference to Related Applications

This application is a continuation of co-pending U.S. Patent Application Serial No. 09/013,375, filed January 26, 1998, which is a continuation of U.S. Patent Application Serial No. 08/454,967 filed May 30, 1995, now abandoned; which is a continuation-in-part of U.S. Patent Application Serial No. 08/146,852, filed November 3, 1993, now abandoned; which is a continuation-in-part of U.S. Patent Application Serial No. 08/095,240, filed July 20, 1993, now

VAL6131P0208A

abandoned; which is a continuation-in-part of U.S. Patent Application Serial No. 07/990,202, filed December 14, 1992, now abandoned; which is a continuation-in-part of U.S. Patent Application Serial No. 07/971,786, filed November 5, 1992, now abandoned.--

Page 6, line 3, please insert --Ia-- beneath the structure which appears on line 2.

Page 13, line 2, please insert --Ia-- beneath the structure which appears on line 1.

Please replace the paragraph beginning at page 20, line 18, with the following re-written paragraph:

--*B. thuringiensis* subsp. *kurstaki* strain EMCC0086 (NB-75, deposited with the NRRL as B-21147) is fermented for 72 hours at 30°C in a medium comprised of a carbon source such as starch, hydrolyzed starch, or glucose and a nitrogen source such as protein, hydrolyzed protein, or corn steep liquor. The production of Ia is detected at 13 hours into the fermentation. Peak activity is found to be at approximately 30 hours.--

Please replace the paragraph beginning at page 23, line 35, with the following re-written paragraph:

--Ia is found to be stable upon boiling for 5 minutes, but loses all activity upon autoclaving (>190°C). Further, it is stable when subjected to direct sunlight for at least 10 hours. Ia is stable at a pH 2 for 3 days, but unstable at pH 12. It is found to lose all activity when exposed to periodic acid or concentrated HCl.--

Please replace the paragraph beginning at page 36, line 23, with the following re-written paragraph:

--Samples of 5 ml are taken daily from each of the shake flask cultures and centrifuged to pellet the cells. The supernatants are diluted 2-10 times in streptomycin at a concentration of 0.1 mg per ml of deionized water and then are tested for antifungal activity as described in Section

7.10.2. Culture samples of those mutants producing the greatest inhibition of fungal growth are then analyzed for the amount of the factor by capillary zone electrophoresis as described in Section 7.10.3. The highest producing mutant from the first mutation is NBB-76.--

Please replace the paragraph beginning at page 39, line 16 with the following re-written paragraph:

--	<u>Strain</u>	<u>Accession Number</u>	<u>Deposit Date</u>
	EMCC0086	NRRL B-21147	October 6, 1993
	EMCC0087	NRRL B-21148	October 6, 1993
	EMCC0129	NRRL B-21445	May 23, 1995
	EMCC0130	NRRL B-21444	May 23, 1995 --

#### IN THE CLAIMS

Please cancel claims 1-17.

Please add new claim 18 as follows:

Claim 18 (new) A biologically pure culture of a mutant of a *Bacillus thuringiensis* subsp. *kurstaki* strain which produces a factor which potentiates the pesticidal activity of a *Bacillus* related pesticide at least about 1.5 fold,

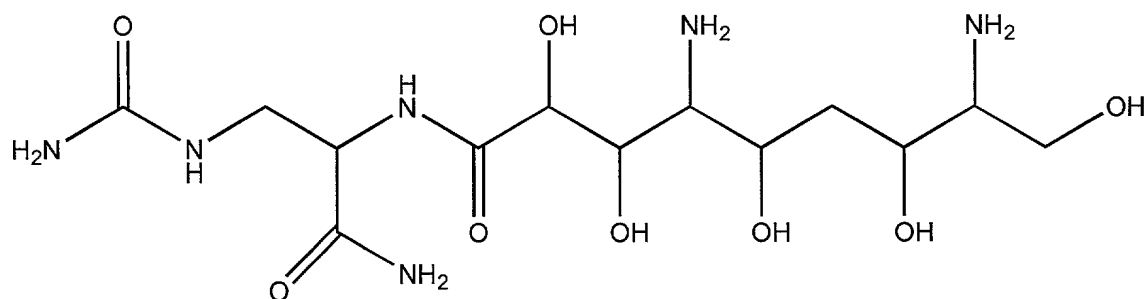
wherein said *Bacillus* related pesticide is a *Bacillus thuringiensis* delta-endotoxin or a pesticidally active fragment thereof and said pesticide targets insects, nematodes, mites or snails,

wherein the amount of the factor produced by the mutant is at least about two times more than the amount of the factor produced by the parent strain,

and wherein said factor has <sup>1</sup>H NMR shifts at about δ 1.5, 3.22, 3.29, 3.35, 3.43, 3.58, 3.73, 3.98, 4.07, 4.15, 4.25 and 4.35, and <sup>13</sup>C shifts at about 31.6, 37.2, 51.1, 53.3, 54.0, 54.4, 61.5, 61.6, 64.1, 65.6, 158.3, 170.7 and 171.3.

Please add new claim 19 as follows:

Claim 19 (new) The mutant of claim 18, wherein said factor has the structure Ia or a salt thereof



Ia .

Please add new claim 20 as follows:

Claim 20 (new) The mutant of claim 18 wherein said *Bacillus thuringiensis* delta-endotoxin or pesticidally-active fragment thereof is selected from the group consisting of CryI, CryII, CryIII, CryIV, CryV and CryVI.

Please add new claim 21 as follows:

Claim 21 (new) The mutant of claim 20 wherein said *Bacillus thuringiensis* delta-endotoxin or pesticidally-active fragment thereof is a CryIA delta-endotoxin or a pesticidally-active fragment thereof.

Please add new claim 22 as follows:

Claim 22 (new) The mutant of claim 20 wherein said *Bacillus thuringiensis* delta-endotoxin or pesticidally-active fragment thereof is a CryIC delta-endotoxin or a pesticidally-active fragment thereof.

Please add new claim 23 as follows:

Claim 23 (new) The mutant of claim 18, wherein said factor is obtained by

- (a) culturing the mutant of said *Bacillus thuringiensis* subsp. *kurstaki* strain to produce said factor;
- (b) recovering a supernatant of the culture of said mutant of step (a); and
- (c) isolating said factor from said supernatant of step (b).

#### Remarks

##### The Present Invention

The invention is directed to a mutant *Bacillus* strain which produces a factor which potentiates the pesticidal activity of a *Bacillus*-related pesticide, a chemical pesticide and/or a virus with pesticidal properties, in which such a factor is obtained in larger amounts or has a greater potentiating activity compared to the parental strain, and methods for producing such mutant strains. The invention also relates to methods for obtaining the factor.

The new set of claims and specification modifications introduced by the present amendment do not constitute new matter, but rather repeats claims presented and modifications to the specification made during the prosecution of the parent application, U.S. Patent Application Serial No. 09/013,375.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The seven attached pages are captioned "Version with Markings to Show Changes Made".

Conclusion

The Applicants respectfully request favorable consideration and allowance of claims 18-23. If any fees are incurred as a result of the filing of this paper, authorization is given to charge Deposit Account Number 04-1644.

Respectfully submitted,

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**CERTIFICATE OF EXPRESS MAIL**

I hereby certify that this Preliminary Amendment and any other documents referred to as enclosed herein, are being deposited in an envelope with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated below and addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

EL820908746  
*Express Mail Label No.*

05/15/01  
*Date of Deposit*

Carla Phillips  
*Typed/Printed Name of Person Mailing  
Correspondence*

Carla Phillips  
*Signature of Person Mailing  
Correspondence*

VAL6131P0208A